

Wide Crosses in the Hydrangeaceae: *Dichroa febrifuga* × *Hydrangea macrophylla*

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Index Words: *Hydrangea macrophylla*, *Dichroa febrifuga*, SSR,
hybrid verification

Significance to Industry: Hydrangeas are among the most popular flowering shrubs, with annual U.S. sales of approximately \$32 million. Their popularity is likely to increase in the future, especially if new and improved cultivars are introduced into the marketplace. This study reports the successful hybridization of *Hydrangea macrophylla* with *Dichroa febrifuga*, a closely related species with flowers that remain blue in the absence of aluminum and persistent metallic blue fruit. This work represents the first step towards incorporating these two ornamental traits into *H. macrophylla*.

Nature of Work: Both *Hydrangea macrophylla* and *Dichroa febrifuga* are members of the family Hydrangeaceae. Previous phylogenetic studies have found *H. macrophylla* to be more closely related to *D. febrifuga* than to other members of the genus *Hydrangea* (2, 8). Blue flower color is the most unique and desirable trait of *H. macrophylla*; however, blue flowers are produced only if adequate aluminum is available to the plant. When grown under high soil pH conditions or in soilless media that has not been supplemented with aluminum, *H. macrophylla* flowers will be pink. In contrast, we have observed blue flowers on *D. febrifuga* plants grown in the absence of aluminum. While *H. macrophylla* has ornamental appeal only while inflorescences are present, *D. febrifuga* produces persistent metallic blue fruit that provide winter interest (1). The objective of this study was to hybridize these two species in order to introduce these two unique ornamental traits from *D. febrifuga* into *H. macrophylla*.

Reciprocal crosses were made during Summer 2005 between *Dichroa febrifuga* (UBC Garden form) and two cultivars of *H. macrophylla*, 'Taube' (2n = 3x = 54) and 'Veitchii' (2n = 2x = 36). These cultivars were chosen because of their difference in ploidy level. Most previous attempts to produce wide hybrids using *H. macrophylla* have required embryo rescue (3, 4, 6) With this evidence in mind, about one-third of the capsules were harvested 63 to 68 days after pollination and embryo rescue via ovule culture was attempted using techniques developed for *H. macrophylla* × *H. paniculata* hybrids (5). The remaining two-thirds of the capsules from the controlled crosses remained on the mother plant for about 20 weeks following pollination. Seed were stored at 4°C for 2 months and sown on a commercial seed propagation mix in October 2005. All progeny of the interspecific crosses were grown in a greenhouse following germination. DNA was extracted from all hybrids and parents. Hybrids were verified using eight SSR markers (7).

Results and Discussion: Hybrids were obtained in all combinations of species and cultivars. Principal co-ordinate analysis (PCO) of the SSR data revealed a two dimensional scatter plot (Fig. 1) that showed clear-cut separation between the hybrids, *D. febrifuga*, and *H. macrophylla* cultivars. Interestingly, both triploid and diploid *H. macrophylla* cultivars successfully hybridized with *D. febrifuga* and reciprocal hybrids were obtained from both *H. macrophylla* parents. *Dichroa febrifuga* and *H. macrophylla* 'Veitchii' hybrids were obtained from both ovule culture and seed germination (Table 1). *Dichroa febrifuga* and *H. macrophylla* 'Taube' offspring were obtained by either ovule culture ('Taube' as the female parent) or seed germination (*D. febrifuga* as the female parent). The close phylogenetic relationship between the two species is confirmed in the production of viable hybrids. Though embryo rescue was effective, it was not necessary because hybrid seed germinated readily. Chromosome counts from hybrid root tip cells were attempted, but results were inconclusive. Preliminary analysis of *D. febrifuga* root tip cells in our lab revealed at least 100 chromosomes.

Hybrids are healthy and vigorous. Once hybrids flower, they will be intercrossed to the other hybrids and back-crossed to the parents. With future generations, new combinations of traits could be anticipated, including the potential for large corymbs of blue flowers (regardless of aluminum availability) that later form persistent blue berries. This study also highlights the usefulness of SSR markers to screen intergeneric *D. febrifuga* and *H. macrophylla* hybrids.

Literature Cited:

1. Hinkley, D. J. 2005. *Dichroa febrifuga*. Horticulture. 102: 79.
2. Hufford, L., M.L. Moody, and D.E. Soltis. 2001. A phylogenetic analysis of Hydrangeaceae based on sequences of the plastid gene *matK* and their combination with *rbcl* and morphological data. Int. J. Plant Sci. 162:835-846.
3. Kudo, N., Y. Kimura, and Y. Niimi. 2002. Production of interspecific hybrid plants by crossing *Hydrangea macrophylla* f. *hortensia* (Lam.) Rehd. and *H. quercifolia* Bartr. through ovule culture. Hort. Res. (Japan) 1:9-12.
4. Kudo, N. and Y. Niimi. 1999. Production of interspecific hybrid plants through cotyledonary segment culture of embryos derived from crosses between *Hydrangea macrophylla* f. *hortensia* (Lam.) Rehd. and *H. arborescens* L. J. Japan. Soc. Hort. Sci. 68:803-809.
5. Reed, S.M. 2000. Development of an in ovulo embryo culture procedure for *Hydrangea*. J. Environ. Hort. 18:34-39.
6. Reed, S.M., G.L. Riedel and M.R. Pooler. 2001. Verification and establishment of *Hydrangea macrophylla* 'Kardinal' × *H. paniculata* 'Brussels Lace' interspecific hybrids. J. Environ. Hort. 19:85-88.
7. Rinehart, T.A., Scheffler, B., and S.M. Reed. 2005. Estimating genetic diversity within the *Hydrangea* genus using molecular markers. Proc. SNA Res. Conf. 50:656-659.
8. Soltis, D.A., Q. Xiang, and L. Hufford. 1995. Relationships and evolution of Hydrangeaceae based on *rbcl* sequence data. Am. J. Botany. 82:504-514.

Table 1. Results of hybridization between two *H. macrophylla* cultivars and *D. febrifuga*.

cross	no. crosses made	no. plants obtained from ovules	no. plants obtained from seed
<i>H. macrophylla</i> 'Taube' × <i>D. febrifuga</i>	83	20	0
<i>H. macrophylla</i> 'Veitchii' × <i>D. febrifuga</i>	132	27	4
<i>D. febrifuga</i> × <i>H. macrophylla</i> 'Taube'	61	0	7
<i>D. febrifuga</i> × <i>H. macrophylla</i> 'Veitchii'	106	24	8

Figure 1. Principal co-ordinate analysis scatter plot showing distinct distribution of *D. febrifuga*, hybrids, and *H. macrophylla* cultivars 'Taube' and 'Veitchii' in the first two principal axes of variation. Points representing 'Veitchii' and 'Taube' intergeneric hybrids include *D. febrifuga* × *H. macrophylla* progeny as well as the reciprocal cross.

